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Amphibian Reproductive Success Within Vernal Pools Associated With The Housatonic River Pittsfield to Lenoxdale, Massachusetts

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WOODLOT ALTERNATIVES, INC.
ENVIRONMENTAL CONSULTANTS

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1.0 INTRODUCTION

The U.S. Environmental Protection Agency (USEPA) is conducting an Ecological Risk Assessment (ERA) for the portion of the Housatonic River and its floodplain beginning at the confluence of the East and West Branches of the River in Pittsfield, Massachusetts, and continuing downstream. The Primary Study Area (PSA) for these investigations is the area between the confluence and Woods Pond Dam in Lenoxdale (Figure 1).

The Housatonic River and its floodplain provide habitat for a wide variety of reptiles and amphibians, including up to 35 species of snakes, turtles, frogs, toads, and salamanders (Woodlot Alternatives, Inc. 2002, Section III, Chapter 4). Breeding amphibians use portions of the river and temporary and permanent pools, called vernal pools, in the floodplain for courtship and egg laying. These areas then support larval amphibians for periods ranging from several weeks to more than a year, depending on the species. These life history traits result in amphibians being in regular contact with sediment and water that are contaminated by polychlorinated biphenyls (PCBs) and other contaminants of concern.

This study was conducted in 1999 to determine if PCB contamination was influencing amphibian reproduction and larval growth and development in the vernal pools of the PSA. Observations were made of amphibian species richness, species abundance, rates and types of gross deformities, breeding behavior and condition, egg laying, hatching success, larval growth and development, and rates of metamorphosis. The study was conducted in support of the ERA because (1) amphibians are a key component of the ecosystem and often represent a significant proportion of the biomass in both terrestrial and aquatic habitats; (2) amphibian survival, development, and reproduction was identified as an Assessment Endpoint for the ERA; (3) several amphibians that occur in the PSA are listed as State-Endangered, Threatened, Special Concern, or Watch List species (see Woodlot Alternatives, Inc. 2002, Section III, Chapter 4); and (4) amphibians may bioaccumulate PCBs and pass them on to other animals in the food chain.

This field-based study was complemented by laboratory studies of wood frog (*Rana sylvatica*) reproduction conducted by Fort Environmental Laboratories (FEL)[1] (FEL 2002)

2.0 STUDY AREA DESCRIPTION

The ecological characteristics of the PSA have recently been described in detail (see Woodlot Alternatives, Inc. 2002, Section II). These studies also included comprehensive descriptions of the amphibian communities of the PSA (see Woodlot Alternatives, Inc. 2002, Section III, Chapter 4).

In general, vernal pools are common within the PSA in the *Transitional Floodplain Forest* natural community type and in a variety of similar habitats within the 10-year floodplain. *Transitional Floodplain Forests* are wooded areas subject to flooding during high water events, and they can be found near the river channel throughout most of the PSA. When floodwaters recede, vernal pools retain water and sediments, providing opportunities for amphibians to breed. Vernal pools also retain water from melting snow and rainfall. Sixty-eight vernal and permanent pools were mapped in the PSA during studies conducted in 1998 (Woodlot Alternatives, Inc. 2002).

Up to 19 amphibians potentially occur within the PSA, and 14 have been confirmed to be present during recent studies. An average of 4.4 amphibian species per pool was recorded in the temporary pools of the PSA, whereas semi-permanent and permanent pools supported 6.0 species per pool (see Woodlot Alternatives, Inc. 2002, Section III, Chapter 4). Wood frogs were one of the most abundant amphibians in the PSA and were the primary focus of the present investigation. Spotted salamanders (*Ambystoma maculatum*) were the most common salamander using vernal pools in the PSA and were the subject of limited specific study during these investigations. Both species are characterized as obligate vernal pool breeding species.

[1] Fort Environmental Laboratories, 1414 South Sangre Road, Stillwater, Oklahoma 74074

3.0 METHODS

Field investigations conducted in 1998, as part of a previous study, focused on locating amphibian breeding habitat in the PSA and characterizing its condition and use by amphibians (Woodlot Alternatives, Inc. 2002, Section III). This information was used to identify representative pools containing wood frogs and spotted salamanders. Sediment samples (0 to 6 inches below ground surface) were collected and analyzed to determine the range of tPCB concentrations in these candidate pools. The results were then used to categorize the pools into three ranges of tPCB concentrations: (1) low (arithmetic mean less than or equal to 1 mg/kg); (2) moderate (1 to 15 mg/kg); and (3) high (greater than 15 mg/kg). The final selection of representative pools was then made using additional information on pool characteristics, including location, proximity to possible disturbances, and hydro-period (Table 1). Later in the study, all of the floodplain data were spatially weighted based upon habitat type to support the ERA, Human Health Risk Assessment, and Modeling Study. These spatially weighted concentrations are also presented in Table 1, and referred to later in the report.

TABLE 1 TOTAL PCB CONCENTRATIONS IN VERNAL POOL STUDY LOCATIONS

Pool	Original Contamination Category	tPCB Levels in Sediment (mg/kg)			
		Minimum	Maximum	Arithmetic Mean (n)	Spatially Weighted Mean
46-VP-5	Low	0.29	3.27	0.99 (11)	0.59
8-VP-1	Moderate	8.3	15.6	11.0 (3)	24.56
38-VP-2	High	4.45	50.65	29.45 (5)	32.31
8-VP-2	High	15.00	154.00	62.1 (10)	54.98

Source: Spatially weighted means provided by The Cadmus Group, Inc., Ottawa, ON, Canada.

The pools selected for this study occurred predominantly in the upper (northern) half of the Housatonic River PSA. Two pools (8-VP-1 and 8-VP-2) were located near the confluence of the East Branch and West Branch of the Housatonic River. One (38-VP-2) was located on the west side of the river just north of New Lenox Road, and the fourth (46-VP-5) was located along the east side of the Springfield Terminal railroad track just north of Yokum Brook, on the west side of the river (Figure 1). Weather was monitored daily at each pool during the field investigations using a minimum-maximum thermometer and a rain gage. Each day, the minimum and maximum temperatures and rainfall experienced during the previous 24-hour period were recorded and the gages were reset. Relative humidity, ambient temperature, and time of measurement were also recorded daily at each pool. Water quality data were recorded daily during the course of the study at each vernal pool and included temperature at the time of visit, pH, dissolved oxygen, and conductivity.

Amphibian reproductive success was evaluated for each stage of the breeding process, including (1) amphibians entering and leaving vernal pools; (2) courtship and breeding behavior and condition; (3) egg laying, hatching success, and larval growth and development; and (4) metamorphosis and exodus from vernal pools (Table 2).

TABLE 2 AMPHIBIAN REPRODUCTIVE SUCCESS STUDY SEGMENTS AND ENDPOINTS

Study Segment	Data Collected	Endpoints
Amphibians Entering and Leaving Vernal Pools	Counts of Animals Entering and Leaving Pools by Species and Sex	• Species Richness and Diversity of Breeding Populations • Sex Ratios of Breeding Populations • Total Biomass by Species
	Body Metrics By Species and Sex	• Body Sizes by Species
	Count of Deformities, Erosion, Lesions & Tumors (DELTs)	• Rates of DELTs
	Marking and Recapturing Animals	• Length of Time Spent in Pools
Courtship and Breeding Behavior and Condition	Audio Surveys of Chorusing	• Breeding Behavior
	Observations of Breeding Activity (e.g., frogs in amplexus)	• Breeding Activity
	Presence of Egg Masses and Spermatophores	• Completion of Breeding
Egg Laying, Hatching Success, and Larval Growth and Development	Observation of Egg Hatching in Box Samplers	• Hatching Success
	Length Measurement of Larvae in Box Samplers (daily for 10 days)	• Early Growth and Development • Early Survival Rates • Rates of DELTs
	Length Measurement of Larvae in Aquatic Funnel Traps (weekly)	• Growth and Development • Rates of DELTs
Metamorphosis and Exodus from Pools	Counts of Metamorphs Leaving Pools by Species	• Counts of Metamorphs per Breeding Female

3.1 AMPHIBIANS ENTERING VERNAL POOLS

Drift fences with pit traps were constructed around each of the study pools (Corn and Bury 1990, Corn 1994, Dodd and Scott 1994)[2] to determine the relative abundance of amphibians entering vernal pools to breed. Pit traps were constructed from two No. 10, or similar, metal cans (6-inch [15.6-cm] diameter, 14-inch [35.6-cm] depth). Traps were placed at 18-foot (5-m) intervals and were paired inside and outside the fence. Each trap pair was given a unique number and its position was recorded using a *Trimble ProXR*™ GPS receiver generally accurate to within 1 to 2 m. Drift fences and traps were installed by 3 April 1999.

Pit traps were checked daily and captured individuals were identified to species, sexed, measured, and weighed (to nearest 0.1 g)[3]. Body measurements (to nearest mm) included head and body length (snout-vent length or SVL), tail length, total length, and the length of each limb.

At least 25 male and 25 female wood frogs, and all spotted salamanders, were marked each day using fluorescent elastomer pigments[4] (see Nauwelaerts *et al.* 2000). Individuals were marked with pigments in colors and locations on the body that were unique to the individual animal, species, sex, and study pool (Donnelly *et al.* 1994). Marked animals were then released on the inside (pool side) of the drift fence. Once animals started to be trapped in pits located inside the drift fence, animals were examined for elastomer marks, weighed, and released on the outside of the fence. In addition to marking and body measurements, each individual captured was inspected for gross external deformities, erosion, lesions, and tumors (DELTs) using procedures developed by the *North American Reporting Center for Amphibian Malformations*.

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[2] Scientific collecting permits for the studies described in this work plan were obtained from the Commonwealth of Massachusetts, Division of Fisheries & Wildlife.

[3] On days when large numbers of animals were captured, a random sample of 25 individuals per species/sex were weighed to avoid stressing the remaining animals with prolonged holding times.

[4] Northwest Marine Technology, Inc., Shaw Island, WA

3.2 COURTSHIP AND BREEDING BEHAVIOR AND CONDITION

Courtship activity and breeding behavior and condition within the study pools were monitored as soon as the first amphibians were captured and placed on the pool side of the fence. Audio surveys were conducted largely to determine if male frogs in the vernal pools were calling to advertise their position to potential mates and rivals (Zimmerman 1994). To conduct these surveys, observers sat quietly beside a pool and noted the general presence or absence of frog choruses.

Notes on any apparent breeding activity were also recorded during daily inspections of the pools. This included observations of frogs in amplexus (the breeding position), the presence of salamander spermatophores, and the presence of egg masses. In addition, animals captured in pit traps were inspected for external indicators of breeding preparedness. Some of these characteristics persist throughout adult life, but others occur in response to gonadotropic hormones and can serve as indicators of reproductive activity (Duellman and Trueb 1986). These types of indicators include (1) swollen and roughened nuptial pads on the inner fingers of male frogs; (2) swollen vents of male salamanders; and (3) generally larger size of egg-laden females.

3.3 EGG LAYING, HATCHING SUCCESS, AND LARVAL GROWTH AND DEVELOPMENT

Within each study pool, up to five wood frog and spotted salamander egg masses for each of the focus species were enclosed in a box sampler to monitor hatching success (Shaffer *et al.* 1994). Box samplers were 20 inches (50 cm) long, wide, and deep and fitted with fiberglass screens on all sides to exclude predators while allowing water and phytoplankton and zooplankton to flow into the sampler. Box samplers were monitored daily to evaluate egg development. At that time, water temperature, dissolved oxygen (DO), pH, and conductivity in the box samplers were recorded. Temperature was recorded automatically using a *HOBO*® data logger[5] and the remaining parameters were measured with hand-held instruments (*YSI*® *85* salinity, conductivity, DO, and temperature system, and *Omega*®

[5] Onset Computer Corp., North Falmouth, MA

PHH-1X pH meter). Similar data were also obtained from permanent stations near the center and edges of the pool, and a staff gage was used to monitor daily water levels in the pool.

Once hatching was complete, the remaining egg gelatin in each box sampler, including hatched, unhatched, and necrotic eggs, was collected and sent to FEL for examination. At FEL, a count of hatched eggs, unhatched fertilized eggs, unfertilized eggs, and necrotic eggs were made for each egg mass.

Following hatching, larval amphibians in the box sampler were monitored daily for 10 days. Larvae were counted, measured for body length (SVL), tail length, and total length, and due to their small size, a counted sample of larvae was weighed to obtain an average weight. To the extent practicable in the field, larvae were inspected for gross DELTs. After 10 days, a random sample of 25 individuals per species, or 50% of the total if fewer than 25 were available, in each box sampler were collected for examination by FEL. The remaining larvae were released into the vernal pool.

Ten aquatic funnel traps were placed in each study pool, beginning the first week of April 1999, to monitor larval growth and development on a weekly basis (Shaffer *et al.* 1994). Traps were placed in pools in the evening and then collected the following morning. At each trap location, water depth and microhabitat information were recorded, as were the trap entry and exit date and time. The total number of larval amphibians of each species were recorded for each trap, as was the SVL, tail length, total length, and length of each limb of up to 25 individuals of each species. For abundant species, such as wood frogs, five measured individuals from each trap were weighed and sent to FEL for examination. When traps contained fewer than 10 individuals, half of the individuals were sent for examination. For less common species, such as spotted salamanders, only one individual was sacrificed from each trap per week. General notes on trap mortality, condition of larvae, associated invertebrates, and any other observations were also recorded.

3.4 METAMORPHOSIS AND EXODUS FROM VERNAL POOLS

Drift fences and pit traps were monitored through the metamorphosis and exodus period for wood frogs, which is generally in mid-July. Captured wood frog metamorphs were counted, measured, weighed, and inspected for DELTs using the methods described above. Following examination, these individuals were placed on the outside of the drift fence. Animals that succumbed to incidental mortality were retained, preserved, and then sent to FEL for examination. Below-normal rainfall during the study period resulted in the drying of vernal pools prior to the full development and metamorphosis of spotted salamanders (see Results, Section 4.0).

4.0 RESULTS

Precipitation during 1999 totaled 39.4 inches (99.1 cm), which is approximately 96% of the 10-year average for the region. During April, however, rainfall was 52% of the 10-year average, and totaled only 1.22 inches[6] (3.1 cm). As a result, 2 of the 4 study pools (8-VP-1 and 8-VP-2) became dry before wood frogs or spotted salamanders could hatch and develop to metamorphosis. Vernal pool 8-VP-1 went dry on approximately 30 April 1999 and did not refill until about 20 May. This pool again went dry on about 10 June 1999, at which time monitoring was discontinued at this location. Vernal pool 8-VP-2 went dry on about 15 April 1999 and remained dry until 22 May. It then went dry again, however, almost 2 weeks later on 3 June 1999.

Vernal pool 38-VP-2 remained wetted through April and May, but dried out for approximately 12 days from 17 June to 29 June 1999. Immediately following re-filling, wood frogs metamorphosed and exited the pool. A similar pattern was observed at pool 46-VP-5.

[6] Source: Commonwealth of Massachusetts data for the Dalton, MA, gage.

4.1 AMPHIBIANS ENTERING VERNAL POOLS

Data from this portion of the study were compared to determine the differences, if any, between (1) the relative abundance of amphibian species using each of the study pools; (2) the time of arrival, by species and sex, between study pools; (3) the proportion of the population with DELTs between pools; and (4) the body sizes of amphibians, by species and sex, among pools. The primary purposes of these analyses were to characterize the community of breeding amphibians within each vernal pool and determine if there were substantial differences between pools with varying levels of tPCBs in the pool sediments.

4.1.1 Relative Abundance of Species

Eleven amphibian species were documented in the four study pools (Table 3). Species richness was greatest in 46-VP-5 (11 species), which had a low level of tPCB contamination, and lowest in 8-VP-2 (5 species), the pool with the highest level of tPCB contamination. Species diversity, as measured by the Shannon-Wiener index (H)[7], was also higher in 46-VP-5.

With the exception of the two Special Concern species noted above, and the lack of spotted salamanders in 8-VP-2, the composition of the community of breeding amphibians was relatively similar across pools. On a per/m^2 basis, however, vernal pool 8-VP-2, the pool with the highest mean tPCB concentration (62.1 mg/kg), had only 9.1% the density of breeding wood frogs of vernal pool 46-VP-5, the pool with a mean tPCB concentration of < 1 mg/kg in the sediments (Table3).

Wood frogs and spotted salamanders are both obligate vernal pool breeding species that occupy similar breeding habitats. To compare the relative abundances of adult wood frogs and spotted salamanders between the four study pools, a chi-square analysis of the number of individuals entering each pool was conducted (see data in Table 3) (Zar 1999:486). The results (chi-square=81.2158, df=7, $p<0.0000$) indicated that the relative abundance of wood frogs and spotted salamanders varied within one or more of the study pools in relation to the

[7] Diversity indices were calculated using the Species Diversity & Richness, version 3.0, software (Pisces Conservation, Ltd, 2002).

other pools. Vernal pool 46-VP-5, which accounted for almost 70% of the chi-square test statistic, was removed from the analysis and the chi-square test was re-run. These results (chi-square=6.0899, df=5, $p<0.2976$) were not statistically significant and confirmed that the number of spotted salamanders observed in pool 46-VP-5, relative to the number of wood frogs, was higher in this pool compared to the pools with greater tPCB contamination.

The lower species richness of salamanders in the pools with the highest levels of tPCBs, and the lack of any salamanders in the pool with the greatest tPCB levels, suggests that this taxonomic group may be sensitive to the concentrations of tPCBs documented in these pools.

TABLE 3 ADULT AMPHIBIANS CAPTURED ENTERING VERNAL POOLS

Pool and Spatially Weighted Mean tPCBs (mg/kg)	**46-VP-5** **0.59**	**8-VP-1** **24.56**	**38-VP-2** **32.31**	**8-VP-2** **54.98**
Salamanders				
Spotted salamander	46	8	10	0
Jefferson salamander	6	0	0	0
Four-toed salamander	2	0	0	0
Red-backed salamander	2	0	0	0
Eastern newt	1	2	1	0
Frogs and Toads				
Spring peeper	2	0	2	0
Green frog	15	11	14	22
Bullfrog	1	4	1	6
Northern leopard frog	24	2	31	13
Wood Frog	283	232	569	167
American toad	6	1	1	2
Total Number of Individuals	**388**	**260**	**629**	**210**
Total Number of Species	**11**	**7**	**8**	**5**
Shannon-Wiener Diversity (H)	**1.022**	**0.503**	**0.439**	**0.737**
Pool Size at Time of Capture (m^2)	**260**	**520**	**630**	**2100**
Individuals/m^2 of Pool	**1.5**	**0.5**	**1.0**	**0.1**
Wood Frogs/m^2 of Pool	**1.1**	**0.4**	**0.9**	**0.1**
Spotted Salamanders/m^2 of Pool	**0.2**	**0.02**	**0.02**	**0**
Wood Frog:Spotted Salamander	**6:1**	**29:1**	**57:1**	**0**

Sex ratios observed in the adult breeding populations of the two primary study species, wood frogs and spotted salamanders, varied between pools (Table 4). The differences between pools for wood frogs were not statistically significant (chi-square=4.6447, df=7, $p<0.7032$), however, and the overall sex ratio for all pools combined was 1.2 male:1 female. Although not statistically different from the other pools, the sex ratios observed in pools 8-VP-1 and 8-VP-2 (0.8 male:1 female and 0.9 male:1 female, respectively) are lower than what is typically seen in breeding wood frog populations (Berven 1990). The overall sex ratio for spotted salamanders (i.e., all pools combined) was 1.6 male:1 female, and the differences between pools were also not statistically significant (chi-square=0.7327, df=5, $p<0.9811$).

TABLE 4 SEX RATIOS (MALE:FEMALE) OF BREEDING ADULT WOOD FROGS AND SPOTTED SALAMANDERS

Pool and Spatially Weighted Mean tPCBs (mg/kg)	46-VP-5 0.59	8-VP-1 24.56	38-VP-2 32.31	8-VP-2 54.98
Spotted salamander	1.5:1	3:1	1.2:1	n/a
Wood frog	1.3:1	0.8:1	1.5:1	0.9:1

The relative biomass (g/m^2) of the two primary study species is shown in Table 5.

TABLE 5 BIOMASS (g/m^2) OF BREEDING ADULT WOOD FROGS AND SPOTTED SALAMANDERS

Pool and Spatially Weighted Mean tPCBs (mg/kg)	46-VP-5 0.59	8-VP-1 24.56	38-VP-2 32.31	8-VP-2 54.98
Spotted salamander	2.5	0.3	0.3	0
Wood frog	10.7	4.5	9.0	0.5

4.1.2 Time of Arrival and Residency Length

There were no apparent differences in the time of arrival between pools for breeding populations of wood frogs or spotted salamanders. Both species arrived at the pools in large numbers during the first 10 days of April, which is typical behavior for breeding populations of both species. The average length of stay of breeding adults in each pool was estimated for wood frogs and spotted salamanders using recoveries of marked individuals (Table 6).

TABLE 6 RESIDENCY LENGTH (DAYS [n]) OF BREEDING WOOD FROGS AND SPOTTED SALAMANDERS AS DETERMINED BY RECOVERY OF MARKED INDIVIDUALS

Pool and Spatially Weighted Mean tPCBs (mg/kg)	46-VP-5 0.59	8-VP-1 24.56	38-VP-2 32.31	8-VP-2 54.98
Spotted salamander				
Males	20.3 (17)	23.3 (6)	12.5 (2)	n/a
Females	15.9 (11)	16.0 (1)	9.0 (2)	n/a
Wood frog				
Males	9.2 (18)	14.8 (19)	11.4 (9)	18.3 (3)
Females	1.0 (5)	8.0 (10)	10.3 (4)	4.8 (5)

4.1.3 Proportion of Populations with DELTs

External gross malformations were observed in only a few adults entering the study pools. One leopard frog in vernal pool 38-VP-2 had a stomach lesion, and a green frog in the same pool was missing a front leg. Both types of injuries can occur from predation attempts. One adult spotted salamander was missing a toe (vernal pool 46-VP-5) and another had fused digits (vernal pool 8-VP-1). With such low gross malformation rates in the adult breeding population, it was not possible to compare pools statistically. Malformations in wood frog larvae are discussed in Section 4.3.

4.1.4 Body Sizes

The weight of male wood frogs entering the pools to breed varied slightly from pool to pool, but differences were related to the length of the individuals entering the pool (ANCOVA, $F(3,199)=2.7125$, $p=0.0461$) (Table 7). Female wood frogs in vernal pool 8-VP-1 weighed more, on average, than individuals captured in pools 8-VP-2 and 46-VP-5. Females in pool 38-VP-2 were also slightly heavier than individuals in 46-VP-5. Even accounting for body length, differences in size for breeding females between pools were statistically significant (ANCOVA, $F(3,125)=7.0950$, $p=0.0002$). These differences, however, are not believed to be biologically significant.

TABLE 7 MEAN WEIGHT (g [se, n]) OF BREEDING WOOD FROGS AND SPOTTED SALAMANDERS

Pool and Spatially Weighted Mean tPCBs (mg/kg)	46-VP-5 0.59	8-VP-1 24.56	38-VP-2 32.31	8-VP-2 54.98
Spotted salamander				
Males	12.8 (0.33, 25)	14.8 (0.67, 6)	12.8 (0.67, 6)	n/a
Females	21.0 (1.49, 17)	34.4 (4.3, 2)	25.6 (3.07, 4)	n/a
Wood frog				
Males	10.9 (0.32, 75)	11.0 (0.36, 59)	11.00 (0.42, 43)	12.6 (0.54, 26)
Females	17.2 (0.43, 46)	20.4 (0.47, 39)	18.8 (0.67, 19)	17.7 (0.58, 26)

4.2 COURTSHIP AND BREEDING BEHAVIOR AND CONDITION

The goals for analyzing data related to wood frogs for this portion of the study included determining if (1) wood frog males were chorusing in an attempt to attract mates; (2) females with eggs were entering the pools to be fertilized; and (3) normal breeding behavior was occurring (e.g., males and females in amplexus). Similar attempts were also made to determine if normal courtship and breeding behavior was taking place for spotted salamanders.

Courtship activities were evident for both wood frogs and spotted salamanders in most of the study pools. The presence of adult animals entering the pools during the breeding period was itself evidence of the initiation of breeding activity. Choruses of male wood frogs were generally heard from 1 April to 8 April 1999 at each pool, also suggesting normal breeding behavior. No such vocal activity takes place for spotted salamanders. Instead, males and females undertake a visual/physical courtship, or nuptial dance, that ends with the male depositing a number of spermatophores on the substrate of the pool. These spermatophore "fields" were relatively easy to observe and were definitive evidence of breeding activity of spotted salamanders, since a male will not deposit them until he performs breeding courtship with a receptive female. Spermatophore fields were observed in all pools except 8-VP-2, in which no spotted salamanders were captured.

4.3 EGG LAYING, HATCHING SUCCESS, AND LARVAL GROWTH AND DEVELOPMENT

The goals in analyzing data related to wood frogs from this portion of the study included determining if wood frogs were depositing egg masses and determining if there were differences in rates of hatching success between pools, larval growth and development between pools, and rates of malformation in developing larvae between pools. Although not studied at the same level of detail, efforts were also made to evaluate egg laying, hatching success, and larval growth and development in spotted salamanders.

Egg masses of wood frogs were observed in all pools, confirming that wood frogs mated and deposited eggs successfully. In addition, egg deposition by spotted salamanders was confirmed in all pools except 8-VP-2. Successful hatching was also observed in all pools for wood frogs, and all pools except 8-VP-2 for spotted salamanders, indicating that eggs had been successfully fertilized in all pools.

Wood frog egg masses that were placed in box samplers were monitored for 10 days following hatching to evaluate short-term survival of newly hatched larvae (Table 8). Egg masses were not put into box samplers in pool 8-VP-2 because there were only a few masses found in the pool and, therefore, there were not enough to conduct that part of the study. Since hatching occurred over a period of several days, it was not possible to calculate a daily survival rate. Comparing the maximum count (i.e., the largest number of individuals observed) in each box, however, with the count after 10 days provides a general comparison of survival between the pools. In general, 10-day survival was low in most of the box samplers (i.e., overall range 0 – 50%), and there were no discernible patterns between pools.

TABLE 8 WOOD FROG 10-DAY LARVAL SURVIVAL IN BOX SAMPLERS

Pool and Spatially Weighted Mean tPCBs (mg/kg)	Box Number	Maximum Count	Count at 10 Days	Percent Survival
46VP5 0.59	1	110	0	0
	2	1040	250	24.0
	3	560	17	3.0
	4	800	4	0.5
	5	7	0	0
8VP1 24.56	1	1000	400	40.0
	2	1080	300	27.8
	3	1200	250	20.8
	4	1120	270	24.1
	5	640	14	2.2
38VP2 32.31	1	840	220	26.2
	2	400	200	50.0
	3	480	175	36.5

Wood frog larvae growth rates during the 10-day period were evaluated by measuring a sample of frogs from each box sampler on a daily basis. The average size of the larvae on the tenth day was used to compare growth rates between pools, and the results indicated that there was more variability between box samplers within a pool than there was between pools, and that there were no biologically significant differences in growth rates between pools (see Table 9).

TABLE 9 MEAN TOTAL LENGTH OF 10-DAY-OLD WOOD FROG LARVAE

Pool Spatially Weighted Mean tPCBs (mg/kg)	Box	N	Mean (mm)	SE of Mean	Duncan's Range[8]	Min	Max
46-VP-5 0.59	4	4	11.18	0.287	A	10.5	11.9
	3	17	11.64	0.156	AB	10.2	12.7
	2	25	12.06	0.122	B	10.7	12.9
8-VP-1 24.56	2	25	10.32	0.091	A	9.3	11.0
	3	25	10.56	0.145	A	9.0	12.7
	4	25	11.35	0.136	B	9.7	12.2
	5	14	11.90	0.233	C	10.2	13.2
	1	25	12.83	0.139	D	11.3	14.3
38-VP-2 32.31	2	25	11.56	0.179	A	8.5	12.8
	1	25	12.03	0.118	B	10.9	12.8
	3	25	12.34	0.185	B	9.7	12.8

Data from larvae collected weekly using aquatic funnel traps were used to compare wood frog growth rates beyond the initial 10-day development period. As noted above, due to the pools drying out in response to low rainfall, wood frog larvae did not successfully develop and go through metamorphosis in pools 8-VP-1 and 8-VP-2. In addition, metamorphosis appeared to be accelerated in pools 46-VP-5 and 38-VP-2 in response to the pools drying out during the larval development period. Limited comparisons were possible, however, between pools 46-VP-5 and 38-VP-2 (Table 10). These results suggest that there were no biologically significant differences in growth rates between these two pools.

FEL examined wood frog larvae from both the 10-day box sampler and weekly aquatic funnel trap (AFT) portions of the study. Malformation rates were relatively high in all pools in the 10-day early development phase, and then throughout the following nine weeks of monitoring (Table 11). The full nature of the types of malformations observed in the PSA has been discussed by Fort (2002).

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[8] Duncan's Multiple Range test was used to compare mean lengths between box samplers *within each pool* after an analysis of variance revealed that there were significant differences ($p<0.05$) between box samplers within each pool. Wood frog growth in boxes with the same letter designation within a pool was not significantly different.

TABLE 10 MEAN WEEKLY BODY LENGTH OF LARVAL WOOD FROGS

Pool & Spatially Weighted Mean tPCBs (mg/kg)	Week	N	Mean (mm)	SE of Mean	Weekly Increase (%)
46-VP-5 0.59	4/27/1999	2	4.45	0.45	--
	5/3/1999	33	5.51	0.10	23.8
	5/12/1999	61	5.71	0.13	3.6
	5/18/1999	32	8.90	0.16	55.9
	5/25/1999	55	9.55	0.22	7.3
	6/2/1999	20	11.32	0.34	18.5
	6/8/1999	65	12.93	0.19	14.2
	6/17/1999	103	13.86	0.15	7.2
	6/22/1999	25	13.13	0.21	-5.3
38-VP-2 32.31	4/27/1999	1	4.20	--	--
	5/3/1999	94	5.38	0.10	28.1
	5/12/1999	81	6.41	0.12	19.1
	5/19/1999	216	8.78	0.09	37.0
	5/26/1999	24	10.29	0.49	17.2
	6/2/1999	37	11.92	0.26	15.8
	6/8/1999	103	13.66	0.20	14.6
	6/17/1999	25	14.32	0.27	4.9
	6/22/1999	25	14.33	0.17	0.1

TABLE 11 MALFORMATION RATES IN LARVAL WOOD FROGS

Pool & Spatially Weighted Mean tPCBs (mg/kg)	Sampler	No. Larvae	No. Malformed[9]	Percent Malformed
46-VP-5 0.59	Box	36	12	33.4
	AFT	270	79	29.3
	Pool Subtotal	*306*	*91*	*29.8*
8-VP-1 24.56	Box	110	53	48.2
	AFT	20	7	35.0
	Pool Subtotal	*130*	*60*	*46.1*
38-VP-2 32.31	Box	51	19	37.2
	AFT	182	40	22.0
	Pool Subtotal	*233*	*59*	*25.3*
Total		**669**	**210**	**31.4**

[9] Source of malformation data is Fort Environmental Laboratories.

4.4 METAMORPHOSIS AND EXODUS FROM VERNAL POOLS

The goals for this portion of the study related to wood frogs included (1) determining if larval forms were successfully transitioning through metamorphosis to the terrestrial life stage; and (2) comparing the number of metamorphs per breeding females between pools.

As noted above, due to the below normal levels of rainfall, metamorphosis did not occur for wood frogs or spotted salamanders in pools 8-VP-1 and 8-VP-2. Although wood frogs successfully completed metamorphosis in pools 46-VP-5 and 38-VP-2, the process appeared to be accelerated in response to low water levels in the pools. Spotted salamanders, however, did not successfully go through metamorphosis in either of these pools.

In pool 46-VP-5, 611 metamorphs were captured leaving the pool (5.0 metamorphs per breeding female entering the pool), compared to 1,113 in pool 38-VP-2 (4.9 metamorphs per breeding female entering the pool). Although these are low numbers of metamorphs per female (Berven 1990), the results were comparable between pools where metamorphosis was successfully completed by some larval wood frogs.

5.0 CONCLUSIONS

The following conclusions were drawn from the results of this study:

- ***Amphibians Entering and Leaving Vernal Pools***
  - Pool 46-VP-5, which had the lowest concentration of tPCBs, appeared to support a richer and more diverse amphibian community than the pools with higher tPCB concentrations.
  - Although not statistically different than the other pools, wood frog sex ratios (male:female) in pools 8-VP-1 (0.8 male:1 female) and 8-VP-2 (0.9 male:1 female) were lower than reported by Berven (1990). These pools contained tPCB concentrations of 24.6 mg/kg and 55.0 mg/kg.

- The density and biomass of breeding wood frogs was lowest in the pool with the greatest tPCB concentration (pool 8-VP-2, 55.0 mg/kg) and highest in the pool with the lowest tPCB concentration (pool 46-VP-5, 0.6 mg/kg).
- Rates of external DELTs were low in all pools for adult frogs and salamanders.

- ***Courtship and Breeding Behavior and Condition***
  - Wood frog and spotted salamander breeding behavior and activity appeared to be outwardly normal in all pools.
  - Wood frog and spotted salamander breeding was proceeding through to fertilization in all pools.

- ***Egg Laying, Hatching Success, and Larval Growth and Development***
  - Wood frogs were fertilizing eggs in all study pools.
  - 10-day growth and development of larval wood frogs was not biologically different between study pools.
  - Larval growth and development of wood frogs during a 9-week development period were not biologically different between study pools.
  - Rates of larval malformation, as determined by laboratory analysis, were relatively high in all pools.

- ***Metamorphosis and Exodus from the Pool***
  - Wood frogs metamorphed in pools 46-VP-5 and 38-VP-2 during a developmental period compressed by low rainfall amounts.
  - The ratio of metamorphs per breeding female was low in both 46-VP-5 and 38-VP-2, but were comparable between pools (ca 5:1).

In sum, species richness and the density and biomass of wood frogs were lower in pools with elevated concentrations of tPCBs. Larval malformation rates were high in all pools potentially indicating that even low levels of tPCBs (0.59 mg/kg in 46-VP-5) may result in increased levels of malformation.

6.0 LITERATURE CITED

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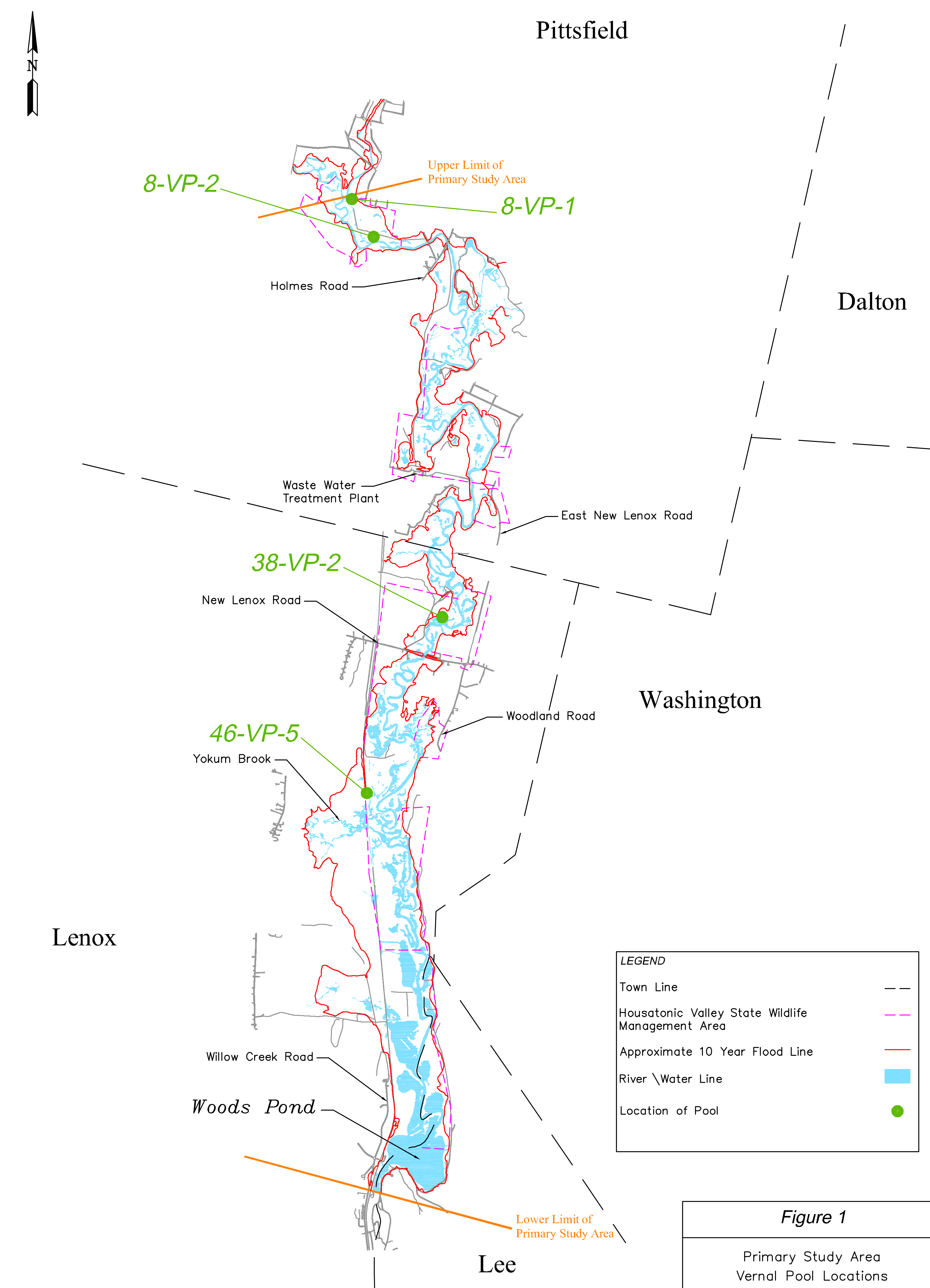
Figures



Feet
3000 0 3000 6000
Meters
1000 0 1000 2000

Figure 1

Primary Study Area
Vernal Pool Locations

Amphibian Reproductive Success
Within Vernal Pools

GE/Housatonic River Project
Pittsfield, Massachusetts

Date: April 2003

Notes:
1) Base Map Information provided by the USEPA.
2) Placement of Town Lines is approximate. Source USGS Quadrangles.
3) Pools surveyed include vernal pools, as defined by the Massachusetts Natural Heritage and Endangered Species Program, and other water bodies that contained, or could contain, breeding amphibians.